A. SCIENTIFIC ABSTRACT

The general goal of this protocol is to determine the safety and feasibility of a gene therapy approach for chronic granulomatous disease (CGD). CGD is an inherited immune deficiency in which blood neutrophils and monocytes fail to produce superoxide and hydrogen peroxide (antimicrobial oxidants), and patients get recurrent life-threatening infections. One third of CGD patients have a defect in the gene that produces $p47^{phox}$ protein required for production of these antimicrobial oxidants. Patients with the $p47^{phox}$ deficient form of CGD will be treated by gene therapy by inserting the cDNA for normal $p47^{phox}$ into hematopoietic progenitor cells (CD34+ cells) using a replication incompetent retrovirus vector. Five patients with $p47^{phox}$ deficient CGD of either sex, at least 13 years of age will be enrolled. Patients will receive six daily morning subcutaneous injections of granulocyte colony stimulating factor (G-CSF) at 10 μg/kg/day to recruit CD34+ cells to the peripheral blood. On days 5 and 6 the patients will have a leukapheresis procedure to harvest 10 to 20 billion peripheral blood mononuclear cells. CD34+ progenitors will be purified 100 fold from the leukapheresis collection using the Isolex 300™ anti-CD34 monoclonal antibody/magnetic bead purification system developed by Baxter Healthcare Corp. CD34+ cells will be cultured in gas permeable plastic bags in defined serum-free medium, X-VIVO 10™ (Bio-Whittaker) containing 1% human serum albumin, and two hematopoietic growth factors, PIXY321 (100 ng/ml, Immunex) and G-CSF (10 ng/ml, Amgen). Cultured CD34+ progenitors will be transduced on each of 3 days with MFG-S-$p47^{phox}$ retrovirus supernatants produced by Somatix Therapy Corp. This is a replication defective retrovirus vector derived from Moloney murine leukemia retrovirus and packaged in the amphotropic envelope packaging line, ψ-crip. At the end of the third transduction the CD34+ cells will be washed and infused into the CGD patient. Safety testing for toxins, sterility, and absence of replication competent retrovirus will be performed on the retrovirus producer lines, virus particle lots, and transduced cells. Blood will be tested for the appearance and persistence of gene therapy corrected cells using functional assays of neutrophil hydrogen peroxide production and polymerase chain reaction assay for detection of MFG-S-$p47^{phox}$ insert in white blood cells. The clinical status of patients will be monitored for any evidence of toxicity. It is hoped that information obtained from this study will permit the development of effective gene therapy for CGD.